MYCORRHIZAE OF SALIX ROTUNDIFOLIA IN COASTAL ARCTIC TUNDRA

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INTRODUCTION

The ability of ectomycorrhizae to increase plant growth and nutrient uptake has been well documented (Marks and Kozlowski, 1973). The advantages that ectomycorrhizae afford plants found in regions characterized by short growing seasons have been discussed by Meyer (1973). Miller and Laursen (1978) described some of the more important aspects of ecto- and endomycorrhizae in US Arctic regions and related tundra plants at Barrow, Alaska might benefit from these associations. In spite of the relative importance of mycorrhizae in arctic tundra, very little research has been conducted in this area. Katenin (1964) stated at the time of his survey of mycorrhizae on arctic tundra plants of the USSR, that the bibliography of works dealing with arctic mycorrhizae consisted of less than ten references. Studies dealing with Alaskan arctic mycorrhizae have concentrated primarily on surveying plant roots for the presence of mycorrhizae, and the subsequent classification of these as being ecto-, ectendo, or endomycorrhizae (Miller et al., 1974).

The purpose of the present study was to conduct an analysis of the ecology and physiology of ectomycorrhizae associated with a single arctic plant species. With this purpose in mind, the dwarf deciduous shrub Salix rotundifolia Trautv. was examined with regard to morphological types of mycorrhizae, numbers of mycorrhizae, and types of fungi with which it could form mycorrhizae.

Low temperature plays a crucial role in the availability of nutrients at Barrow, Alaska (Ulrich and Gersper, 1978). Studies were undertaken to elucidate the physiological responses of S. rotundifolia mycorrhizae to temperature. In order to more clearly elucidate the functional role of S. rotundifolia mycorrhizae, their ability to hydrolyze the substrates cellulose and p-nitrophenyl phosphate were examined.

MATERIALS AND METHODS

Root tip counts for S. rotundifolia were obtained during the summer of 1977 from cores 2.5 cm in diameter taken to a depth of 8 cm. Samples collected at Barrow and Cape Simpson were treated as described by Antibus and Linkins (1978). Viable mycorrhizae were counted according to the criteria set forth in Harvey et al. (1976).

Roots employed in respiration studies were collected and handled in a manner similar to those utilized for root tip counts. Respiration data were obtained using a refrigerated Gilson Differential Respirometer as described by Linkins and Antibus (1978). Temperature profiles of oxygen uptake by roots from Barrow and Cape Simpson were run at 26,21,16,10,5,3, and 0.5 C.

Cellulase activities, as endo- and exocellulase components, were determined and expressed as described by Miele and Linkins (1978) and Linkins et al. (1978). Activity of B-glucosidase was determined as described by Berghem and Pettersson (1974). Mycorrhizae and rhizomorphs were isolated from soil in a manner similar to that used for mycorrhizae employed in respiration studies. Pure culture isolates of mycorrhizal fungi were obtained and grown as described by Antibus (1980). All enzymes were assayed in the total culture medium and in 25 mM phosphate buffer (pH 6.1) or in 10 mM Tris.HCl buffer (pH 8.1) in 1.0 M NaCl fortified extracts of mycelia. Both culture media and mycelial extracts were dialyzed to the appropriate assay buffer using an Amicon UM-25 membrane system. Mycelia were homogenized in a Brinkman polytron at full speed for three 20 sec bursts at 0 C. Extraction periods were separated by 60 sec intervals.

Attempts were made to isolate suspected mycorrhizal fungi during both the summers of 1976 and 1977, at Barrow and Cape Simpson, Alaska. Procedures utilized to obtain cultures from fungal fruiting bodies found near Salix plants, and the spores discharged by these fungi, were those described by Palmer (1971). Isolation attempts from surface sterilized roots were those described by Antibus (1980).

In order to test for their ability to form ectomycorrhizae, each fungus was grown in the presence of S. rotundifolia cuttings using the peat-vermiculite technique of Marx and Zak (1965), as outlined by Antibus (1980). After 180 days, roots were harvested and prepared for light microscopy as described by Antibus (1980). Roots examined by scanning electron microscopy (SEM) were prepared by the procedure of Kinden and Brown (1976).

Acid phosphatase activity of mycorrhizae resynthesized under controlled conditions was measured at pH 4.7 in citrate buffer with p-nitrophenyl phosphate used as a substrate in accordance with the procedures of Bartlett and Lewis (1973). Activity estimates were established on a root surface area basis.

RESULTS AND DISCUSSION

ROOT TIP COUNTS

Root tip counts were conducted over one field season at two sites in order to quantify the differences existing between the types of ectomycorrhizae found predominantly at Barrow and Cape Simpson. To expedite the counting procedure, root tip counts were broken down into only two categories, those formed by Cenococcum geophilum Fr. and those formed by fungi other than C. geophilum. Shown in Figure 1 are the results of root tip counts from the 1977 field season. The number of viable S. rotundifolia mycorrhizae was highest at Barrow during early July, but declined steadily during late July and early August. The highest mean value of approximately 20 mycorrhizae per ml of soil was obtained on July 19, while the lowest value of 11 was recorded on August 9. An overall season mean value of approximately 15 mycorrhizae per ml of soil was obtained at Barrow. At this site the number of mycorrhizae formed by C. geophilum relative to the total number of S. rotundifolia mycorrhizae ranged from 7 to 30 percent, with a mean value of 18 percent. This value is equivalent to an average of less than three C. geophilum mycorrhizae per ml of soil.

Counts of viable mycorrhizae at Cape Simpson declined from approximately 31 tips per ml of soil on July 15 to approximately 14 on August 1 (Fig. 1). The overall mean at Cape Simpson was approximately 23 mycorrhizae per ml of soil. This value was about 32 percent higher than that obtained for Barrow. At Cape Simpson the number of C. geophilum mycorrhizae ranged from 31 to 63 percent of the total mycorrhizae, with a mean for all samples of 54 percent. This value would translate to an average of 12 C. geophilum mycorrhizae per ml of soil.

The findings of the present study, which indicates an early to mid season peak in the number of viable mycorrhizae, are in agreement with those of Harvey et al. (1978) for a Douglas fir/larch forest. These authors found that the number of mycorrhizae peaked during a period from May to June and declined on either side of this time period. In addition, they reported that 95 percent of the active mycorrhizal root tips were found in a period from May to October.

Fig. 1.

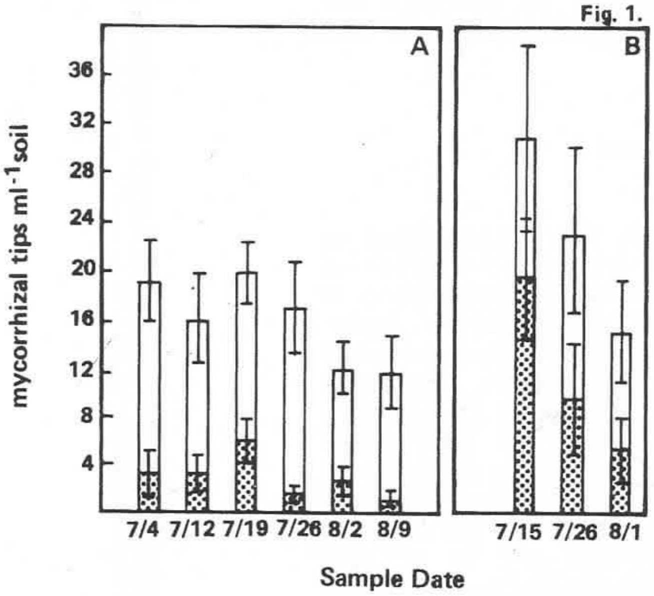
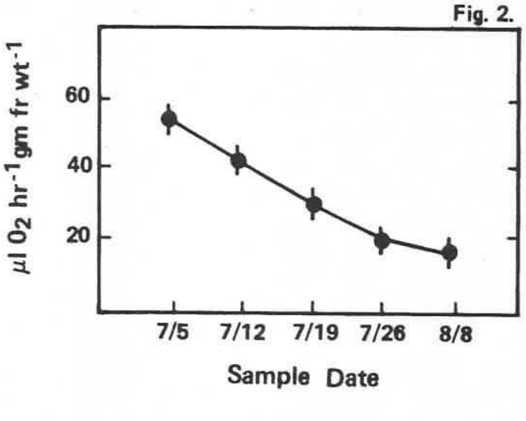

A
B
mycorrhizal tips ml^{-1}soil
36
32
28
24
20
16
12
8
4
7/4
7/12
7/19
7/26
8/2
8/9
7/15
7/26
8/1
Sample Date

Fig. 2.


μl O$_2$ hr^{-1}gm fr wt^{-1}
60
40
20
7/5
7/12
7/19
7/26
8/8
Sample Date

The results of the present study also indicate that the number of mycorrhizal root tips and the percentage of these root tips colonized by C. geophilum was higher at Cape Simpson than at Barrow. These observed differences in the relative numbers of mycorrhizae at the two sites may be due to differences in the soil properties of the two sites. A relationship between root numbers and soil properties has frequently been hypothesized to exist (Meyer, 1964). It is the availability of nutrients and water which are felt to be responsible for the number of root tips that develop, and for the percentage that become mycorrhizal. Salix rotundifolia at the Cape Simpson oil seep, are found growing in very asphaltic and hydrophobic soils. Chemical and physical properties of these soils would favor a fungus like C. geophilum, which is reported by a number of investigators to be an extremely drought tolerant pioneer fungus (Worley and Hacskaylo, 1959; Meyer, 1964; Mexal and Reid, 1973). In addition, these soil conditions may also account for the increased numbers of root tips observed at Cape Simpson.

RESPIRATION

A profile of the respiration rates of S. rotundifolia mycorrhizae obtained over the 1977 growing season at Barrow is shown in Figure 2. These data demonstrate that a progressive decrease occurs in the respiratory rate of these mycorrhizae from early July into early August. This decrease in respiratory rate paralleled the decrease observed over this time period in the number of viable mycorrhizae at Barrow. Taken together, these data indicate that not only do the number of viable mycorrhizae decrease as the growing season progresses, but those mycorrhizae that remain viable are less active as well. These overall patterns of mycorrhizal numbers and respiration rates correlate well with the observed aboveground phenology of S. rotundifolia.

Arrhenius plots relating rates of oxygen uptake to temperature for S. rotundifolia ectomycorrhizae from Barrow and Cape Simpson are shown in Figure 3. Roots from both sites responded in a linear fashion over the temperature range of 26 to 0.5 C. The absolute oxygen uptake values were quite similar for roots from both sites, as were the slopes of the Arrhenius plots.

Linear Arrhenius plots, as observed in Figure 3, are usually taken as an indication that the activation energy of a metabolic process remains constant over the measured temperature range. Sudden shifts or breaks in the slopes of Arrhenius plots are generally associated with increased activation energies and increased Q_{10} values at lower temperatures (Kumamoto et al., 1971; Raison et al., 1971;

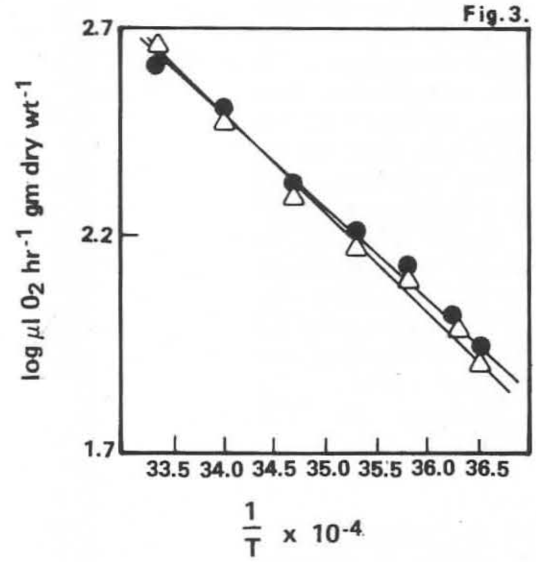

2.7
2.2
1.7
log μl O_2 hr⁻¹ gm dry wt⁻¹
33.5
34.0
34.5
35.0
35.5
36.0
36.5
1/T x 10⁻⁴

Fig.3.

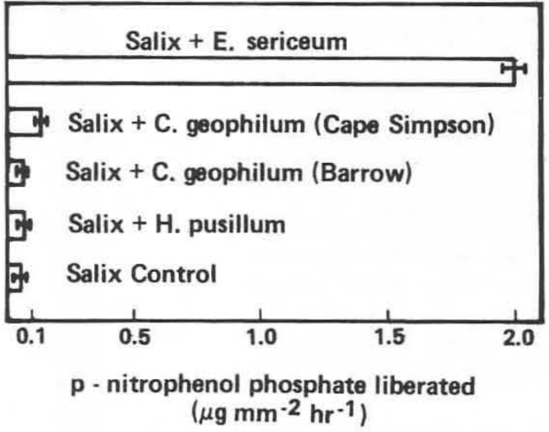

Salix + E. sericeum
Salix + C. geophilum (Cape Simpson)
Salix + C. geophilum (Barrow)
Salix + H. pusillum
Salix Control
0.1
0.5
1.0
1.5
2.0
p - nitrophenol phosphate liberated
(μg mm⁻² hr⁻¹)

Fig. 4.

Raison, 1973). This increase in activation energy for membrane bound enzymes has been attributed to a conformational change caused by a temperature-induced phase-change in membrane lipids (Raison, 1973). Plants susceptible to injury at low temperatures (chilling sensitive) exhibit breaks at various temperatures in Arrhenius plots of respiratory activity and membrane bound enzyme activity (Lyons and Raison, 1970; Lyons, 1973). Plants which are not susceptible to injury at low temperatures (chilling insensitive) exhibit linear Arrhenius plots of respiration and membrane bound enzyme activity. On the basis of field studies, the ectomycorrhizae of S. rotundifolia from Barrow and Cape Simpson would be considered to be chilling insensitive over the temperature range examined. This ability to maintain a constant temperature coefficient for respiration, and thus maintain functional integrity of metabolism over a wide range of temperatures, would prove extremely beneficial to the plant in coastal tundra soils where large daily variations in temperature have been observed to occur (Miller, 1976).

CELLULASE ACTIVITIES

The hydrolysis of cellulose and other recalcitrant plant polysaccharides to glucose for microbial uptake and metabolism is accomplished by a family of cellulase enzymes. These enzymes differ in their activities by the location of the B-1-4 glycoside bonds in the cellulose polymer that they hydrolyze. All microorganisms studied to date, which can utilize cellulose metabolically, produce these enzymes extracellularly. The basic functional groups within this complex are the endocellulases, which hydrolyze bonds located internally within the cellulose polymer; the exocellulases, which hydrolyze bonds at or near the non-reducing end of the polymer; and the B-glucosidases, which hydrolyze the terminal bond at the non-reducing end of the polymer releasing free glucose. All of these enzymes function together to most efficiently convert insoluble cellulose to free glucose for microbial assimilation.

Examination of mycorrhizae and associated rhizomorphs from the Barrow sites showed that the rhizomorphs not only had the highest overall cellulase activity, but also had the highest exocellulase/endocellulase ratio (0.1) suggesting a much more efficient complex for solubilizing cellulose to assimulatable substrate (Table 1). The 150x higher B-glucosidase activity of rhizomorphs further confirms that this cellulase complex is functionally involved in producing glucose from cellulose. The actual potential utilization of this as a carbon source for mycobiont oxidation to CO_2 has been confirmed from $^{14}CO_2$ generation from ^{14}C-cellulose incubated with these mantle and rhizomorph samples Linkins and Antibus (1979).

TABLE 1. Cellulase activity in field collected ectomycorrhizae, associated rhizomorphs, and uncolonized roots of S. rotundifolia. Endocellulase activity (units $hr^{-1}mg^{-1}$ dry weight); exocellulase activity (ug glucose equivalents $hr^{-1}mg^{-1}$ dry weight); B-glucosidase activity (ug PNP liberated $hr^{-1}mg^{-1}$dry weight). Standard deviations in parentheses, n=7.

	Endo-	Exo-	B-glucosidase
Mantle	10.0(1.5)	0.07(0.05)	0.02(0.01)
Rhizomorph	42.4(3.2)	4.2(0.5)	2.8(0.4)
Uncolonized root	3.5(0.5)	0.03(0.01)	0.02(0.01)

Suppression of total cellulase activity and reduction in the exo- and endocellulase ratio (0.1) in the mantle suggests that regulation of the fungal cellulase potential is occurring. The known suppression of cellulase synthesis by glucose, an end product of cellulase activity (Reese, 1977), suggests that the plant carbon translocation of photosynthate to the plant root and fungal mantle (Hacskaylo, 1973) may be the primary regulating factor.

The validity of these findings, as a true expression of the cellulolytic capacity of these mycobionts and not that of associated organisms, was tested with pure cultures of known mycobionts associated with S. rotundifolia. Production of cellulase in cultures with cellulose or glucose as the primary carbon source is shown in Table 2.

Cellulase activity is present in cultures with cellulose and has a functional exo- and endocellulase ratio equal to or greater than those from rhizomorphs (0.11 for Hebeloma and 0.22 for Cenococcum). The presence of glucose in the culture completely represses both endo- and exocellulase activities. This effective glucose supression of cellulase activity supports the [illegible] root's role as a carbon source to the ma [illegible] factor in regulation of cellulase expression by the mantle fungus. These data may also give insight into some of the contradictory

TABLE 2. Cellulase activity in cultures of the S. rotundifolia mycobionts C. geophilum and Hebeloma pusillum J. Lange when grown with cellulose as the primary carbon source. Endocellulase activity (units hr^{-1}mg^{-1}dry weight); exocellulase activity (ug glucose equivalents hr^{-1}mg^{-1} dry weight); B-glucosidase (ug PNP liberated hr^{-1}mg^{-1}dry weight). Standard deviations in parentheses, n=4.

	Endo-	Exo-	B-glucosidase
		Cellul~~o~~ase	
H. pusillum	42.0(2.5)	4.7(0.5)	1.2(0.5)
C. geophilum	12.0(1.4)	2.7(0.6)	0.5(0.2)
		Glucose	
H. pusillum	0.90(0.10)	- 0 -	0.95(0.10)
C. geophilum	0.08(0.05)	- 0 -	0.32(0.20)

literature on the cellulolytic capacity of cultured mycobionts, especially where glucose has been used as a culture "starter".

The presence of B-glucosidase activity, even in the glucose cultures does, however, suggest that the use of cellobiose or other soluble cellooligosaccharides may play a role in mycobiont-carbon assimilations, as supported by the work of Hacskaylo (1975) and Palmer and Hacskaylo (1970). Even though the total cellulase activities reported here are lower than many of the studied obligate saprophytic fungi (Eriksson, 1978; Miele and Linkins, 1978; Reese, 1977), the presence of a functional enzyme complex, constitutive B-glucosidase activity, and the repressible endo- and exocellulase components suggest that cellulolytic activity, compatible with successful mantle and Hartig Net development, can provide some fraction of the carbon required for the mycobiont's growth. Arctic mycorrhizal systems may be more efficient than temperate systems in that they provide increased nutrient absorptive surface area while also providing some carbon for the non-mantle hyphae.

PHOSPHASTASE ACTIVITIES OF RESYNTESIZED ECTOMYCORRHIZAE.

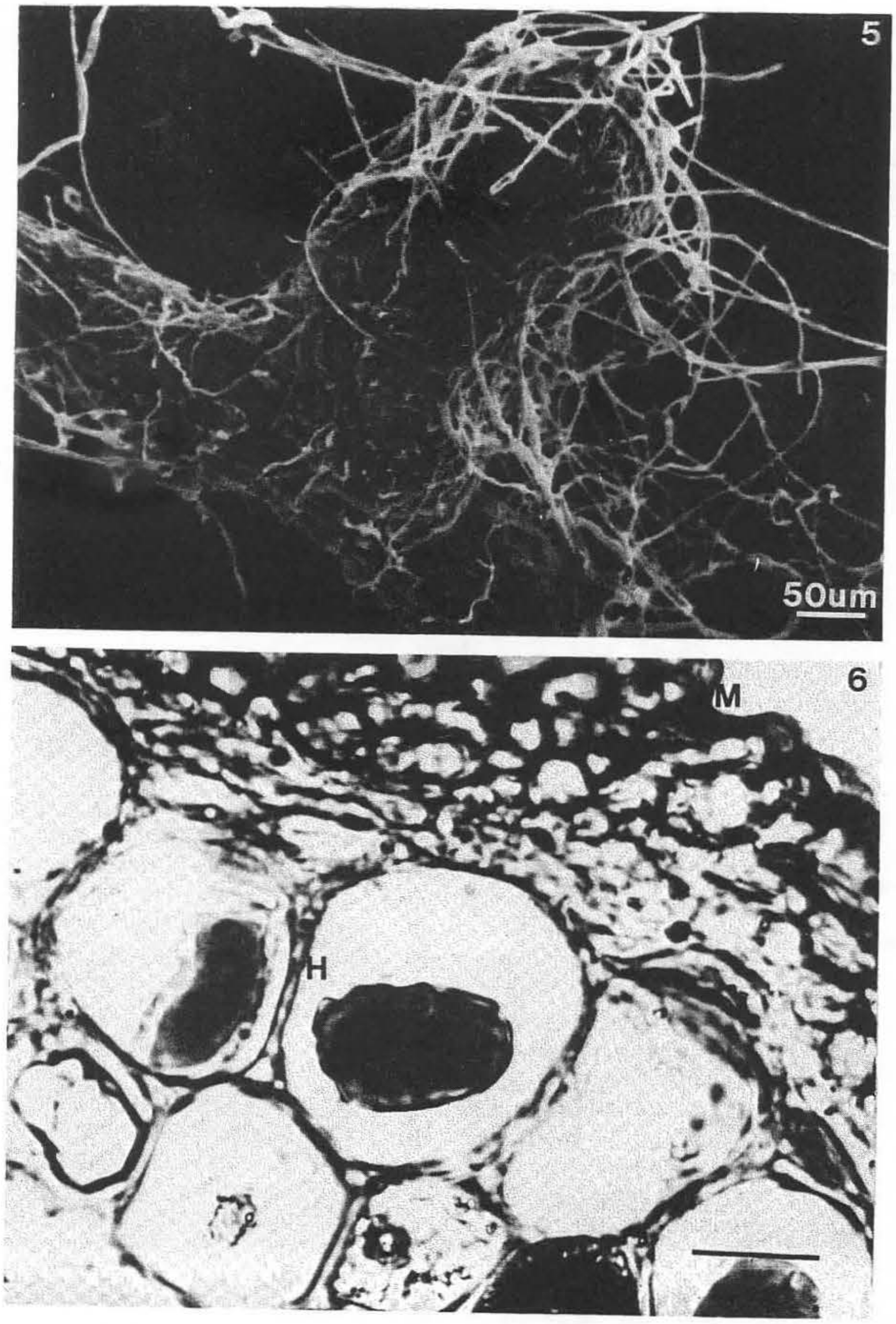
To date most reports regarding mycorrhizal fungi of dwarf willows in arctic regions have been based entirely on field observations. Such observations do not provide definitive proof that a fungus is mycorrhizal with a certain plant (Zak, 1973). It was the desire, through this aspect of the study, to obtain definitive proof as to which fungi could form ectomycorrhizae with *S. rotundifolia* by means of resynthesising studies.

A list of fungi found associated with dwarf *Salix* species, and from which isolation attempts were made over a two year period, is given in Table 3. In all, four isolates were eventually tested for their ability to form ectomycorrhizae with *S. rotundifolia*. These were isolates of *C. geophilum* from Barrow and Cape Simpson, an isolate of *H. pusillum* from Barrow, and an isolate of *Entoloma sericeum* (Bull. ex Merat) Quelet. from Barrow. All four isolates tested proved capable of forming typical ectomycorrhizae with *S. rotundifolia*. Those formed by *C. geophilum* isolates were indistinguishable from those formed in the field by this fungus (Figs. 5 and 6). Both *H. pusillum* and *E. sericeum* formed white ectomycorrhizae superficially similar to each

TABLE 3. Fungal species were found fruiting in the vicinity of dwarf willows at Barrow or Cape Simpson, Alaska during the summers of 1976 and 1977.

-	*Cortinarius subtorvus* Lamoure
+	*C.* aff. *mucosus* (Bull. ex Fr.) Kickx.
-	*C.* aff. *huronensis* var. *huronensis* Ammirati
++	*Entoloma sericeum* (Bull. ex Merat) Quelet.
++	*Hebeloma pusillum* J. Lange
+	*Hebeloma sp.*
-	*Laccaria laccata* (Fr.) Berk. and Br.
-	*L. striatula* (Peck) Pk.
+	*Lactarius lanceolatus* Miller and Laursen in Miller, Laursen and Murray
-	*Russula emetica* var. *alpestris* (Boud. in Boud. & E. Fisch.)Sing.
-	*R. xerampelina* Schaeff. ex Fr. var. *pascua* Moller and J. Schaeff.

-	no growth in pure culture
+	limited growth
++	substantial growth

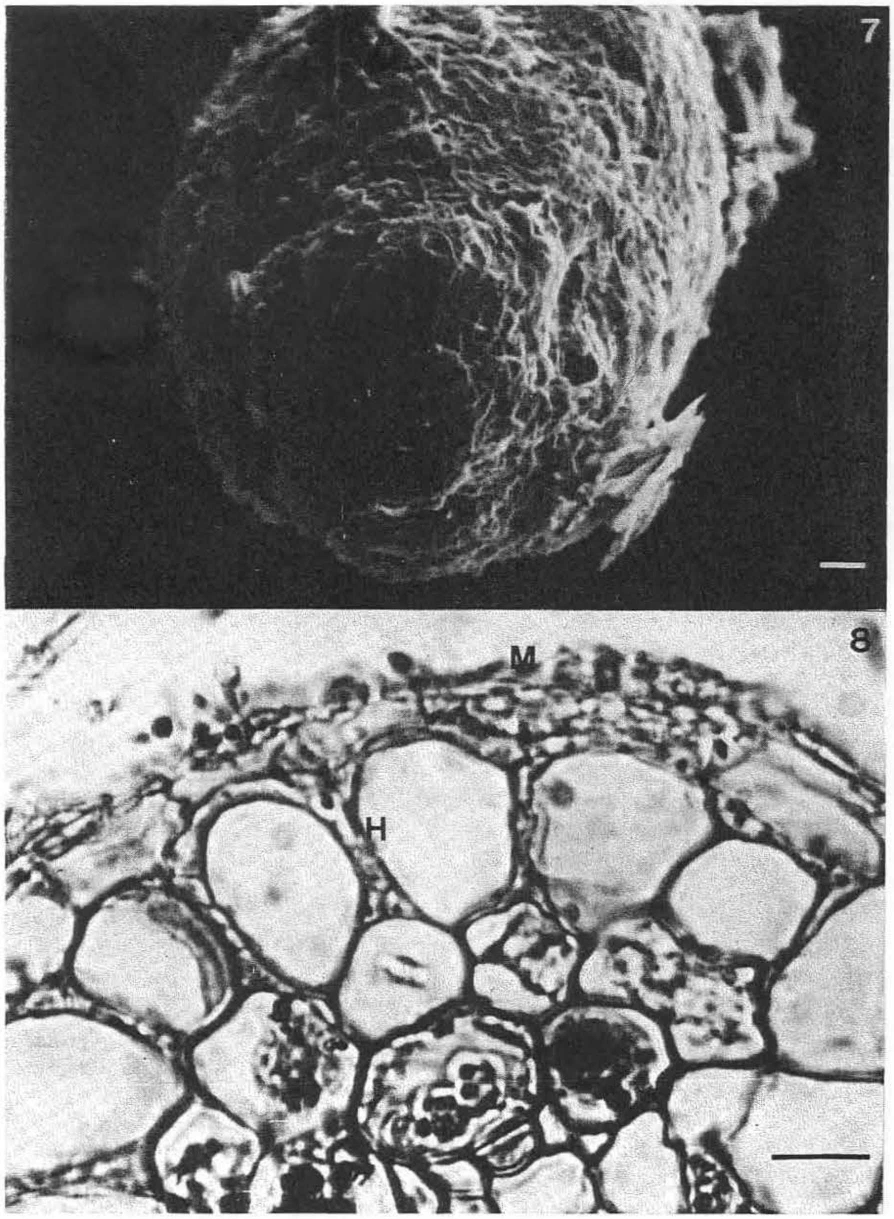


Figures 5-6. Fig. 5. Scanning electron micrograph of mycorrhiza synthesized between Salix rotundifolia and Cenococcum geophilum from Cape Simpson, Alaska; Fig. 6. Cross section of ectomycorrhiza synthesized between S. rotundifolia and C. geophilum, showing the fungal mantle (M) and Hartig net (H). Reference bars represent 10 micrometers unless otherwise indicated.

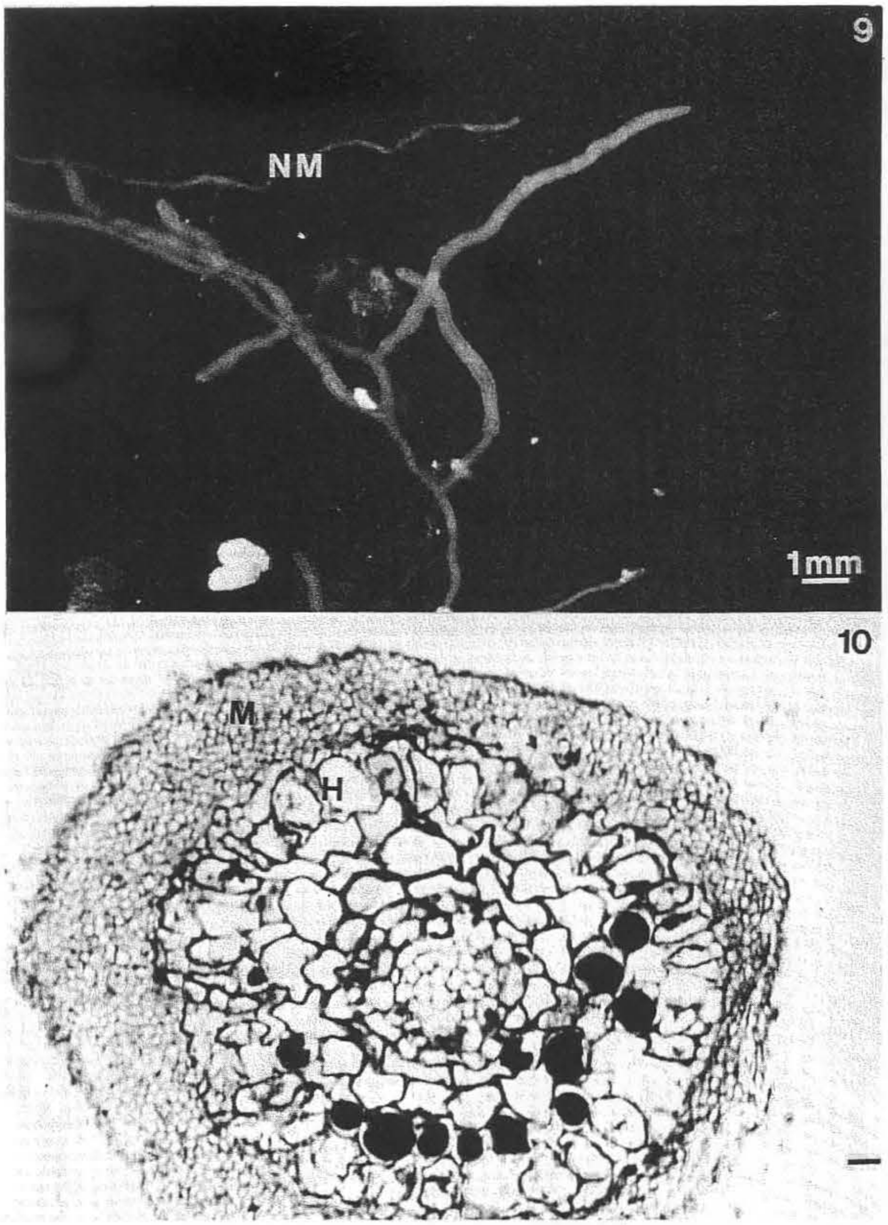
other in appearance (Figs. 7-10). These mycorrhizae could be easily separated by a number of microscopic features such as mantle thickness and hyphal arrangement in the mantle. These mycorrhizae have been described in detail by Antibus (1980).

The comparative abilities of various host-mycobiont combinations to hydrolyze p-nitrophenyl phosphate at pH 4.7 are summarized in Figure 4. It is apparent from these results that the acid phosphatase activity of *E. sericeum* was significantly greater than that of other ectomycorrhizae or uncolonized roots. In fact, the colonization of *S. rotundifolia* roots by *E. sericeum* resulted in a twenty to forty fold increase in acid phosphatase activity over the other roots examined. Williamson and Alexander (1975) had previously reported that infection of beech roots by an unidentified mycobiont resulted in an eight fold increase in acid phosphatase activity. The fact that different ectomycorrhizal fungi differ in their potential to hydrolyze p-nitrophenyl phosphate when grown in pure culture, has also been demonstrated by Ho and Zak (1979). The results of the present study have confirmed their findings, using different ectomycorrhizal fungi in association with a single host species.

These observed differences in acid phosphatase activity might be explained in part by the morphologies of the various associations. Mycorrhizae formed by *H. pusillum* demonstrated the lowest acid phosphatase activity of all the mycorrhizae. This association also formed a very thin mantle over the root surface. Its mean mantle thickness of 9 µm was the lowest of all the fungi tested. In addition, *H. pusillum* mycorrhizae tended to have less attached mycelium than other mycorrhizae examined (Antibus et al., in press). Roots colonized by *C. geophilum* tended to show higher phosphatase activities than controls of *H. pusillum* mycorrhizae. These roots had a thicker mantle, with a mean thickness of 18 µm, and tended to have more attached mycelium (Fig. 5) than by *H. pusillum*. Mycorrhizae formed by *E. sericeum* showed tremendous increases in acid phosphatase activity over control roots and other mycorrhizae. This association also formed the thickest mantle, with a mean of 22 µm, and also displayed the greatest amount of attached mycelium. The quantity of this attached mycelium was not accounted for by surface area measurements; therefore, the phosphatase activity of *E. sericeum* roots has probably been overestimated to some extent. Still, it seems unlikely that the differences observed between the phosphatase activities of *C. geophilum* and *E. sericeum* mycorrhizae can be explained solely on the basis of surface area.



Figures 7-8. Fig. 7. Scanning electron micrograph of mycorrhiza synthesized between Salix rotundifolia and Hebeloma pusillum; Fig. 8. Cross section of S. rotundifolia and H. pusillum ectomycorrhiza.



Figures 9-10. Fig. 9. Ectomycorrhizae synthesized between *S. rotundifolia* and *Entoloma sericeum* along with a nonmycorrhizal (NM) *S. rotundifolia* root; Fig. 10. Cross section of *S. rotundifolia* and *E. sericeum* ectomycorrhiza.

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